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SEARCH REQUEST FORM

22686

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Serial Number: 09/268,989 Results Format Preferred (circle): PAPER DISK E-MAIL

Title of Invention Device for Detecting Molecules, Method for detecting molecules.

Inventors (please provide full names): Fred Stevens, Marianne Shuffer, Priscilla Wilkins-Stevens, W. Corey Hardy, Sandra L. Tollaksen

Earliest Priority Date: 8/5/99

Keywords (include any known synonyms registry numbers, explanation of initialisms):

Please see attached claims & data sheet.

Thanks, :)
Lisa C

Search Topic:

Please write detailed statement of the search topic, and the concept of the invention. Describe as specifically as possible the subject matter to be searched. Define any terms that may have a special meaning. Give examples of relevant citations, authors, etc., if known. You may include a copy of the abstract and the broadcast or most relevant claim(s).

934
917-27

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_____ Westlaw
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PTO-1590 (2-99)

Cook
368989

```
L1          164 FILE MEDLINE
L2          338 FILE CAPLUS
L3          160 FILE BIOSIS
L4          152 FILE EMBASE
L5           40 FILE WPIDS

TOTAL FOR ALL FILES
L6          854 MOLECULE AND ANTIGEN BIND? SITE?

=> s l6 and end termin? and amino moiety and carbonyl moiety

L7            0 FILE MEDLINE
L8            0 FILE CAPLUS
L9            0 FILE BIOSIS
L10           0 FILE EMBASE
L11           0 FILE WPIDS

TOTAL FOR ALL FILES
L12           0 L6 AND END TERMIN? AND AMINO MOIETY AND CARBONYL MOIETY

=> s light chain and l6 and amino and carbonyl

L13           0 FILE MEDLINE
L14           0 FILE CAPLUS
L15           0 FILE BIOSIS
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L17           0 FILE WPIDS

TOTAL FOR ALL FILES
L18           0 LIGHT CHAIN AND L6 AND AMINO AND CARBONYL

=> s l6 and light chain

L19          20 FILE MEDLINE
L20          48 FILE CAPLUS
L21          16 FILE BIOSIS
L22          13 FILE EMBASE
L23           7 FILE WPIDS

TOTAL FOR ALL FILES
L24         104 L6 AND LIGHT CHAIN

=> s juxtapos? or counterpoise?) and l24

UNMATCHED RIGHT PARENTHESIS 'NTERPOISE?) AND'
The number of right parentheses in a query must be equal to the
number of left parentheses.

=> s (juxtapos? or counterpoise?) and l24

L25           0 FILE MEDLINE
L26           0 FILE CAPLUS
L27           0 FILE BIOSIS
L28           0 FILE EMBASE
L29           0 FILE WPIDS

TOTAL FOR ALL FILES
L30           0 (JUXTAPOS? OR COUNTERPOISE?) AND L24
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=> s l24 and (bind? or non bind? or vl domain or amino acid or carbonyl)

L31 20 FILE MEDLINE
L32 48 FILE CAPLUS
L33 16 FILE BIOSIS
L34 13 FILE EMBASE
L35 7 FILE WPIDS

TOTAL FOR ALL FILES
L36 104 L24 AND (BIND? OR NON BIND? OR VL DOMAIN OR AMINO ACID OR CARBON
YL)

=> s l36 and (device or detect?)

L37 4 FILE MEDLINE
L38 7 FILE CAPLUS
L39 4 FILE BIOSIS
L40 4 FILE EMBASE
L41 1 FILE WPIDS

TOTAL FOR ALL FILES
L42 20 L36 AND (DEVICE OR DETECT?)

=> dup rem l42

PROCESSING COMPLETED FOR L42
L43 10 DUP REM L42 (10 DUPLICATES REMOVED)

=> d 1-10 cbib abs

L43 ANSWER 1 OF 10 CAPLUS COPYRIGHT 1999 ACS
1998:128756 Document No. 128:216272 Attractor control of the **binding** of digoxin to a specific antibody. Havsteen, B. (Department of Biochemistry, School of Medicine, University of Kiel, Kiel, D-24098, Germany). J. Theor. Biol., 189(4), 367-376 (English) 1997. CODEN: JTBIAP. ISSN: 0022-5193. Publisher: Academic Press Ltd..
AB The characteristics of attractor control of the changes in the **mol** . vibrations of a protein have previously been **detected** when an enzyme (chymotrypsin) reacted with a specific substrate and when myoglobin interacted with oxygen. Similar studies have now been carried out on the **binding** of a hapten, digoxin, to an antibody. The temp. factors of the Fab-fragment of a specific anti-digoxin antibody with and without the bound antigen were used in this anal. The integral correlation function of the difference in the temp. factor between the free and the loaded state of the **antigen binding site** indicated the existence of a regular attractor of the dimension 4.0 in the **light chain** and one of the dimension 5.7 in the heavy chain, the former under the control of 11 factors and the latter by 12 factors. This result was corroborated by Poincare plots showing the cross-section of attractors and by a pos. Liapunov exponent. The power spectrum was, as expected, broad, but the autocorrelation function showed only significant damping in the case of the L-chain. The spacing of the temp. factors resembled a "Devil's Staircase" suggesting the operation of a stochastic attractor. Its dimension, which was detd. by the methods of the correlation between the step-gap lengths and that of the Farey tree was found to be near one. Repetition of the calcn. using data for the second antigen-antibody complex in the unit cell yielded similar results.

However, the dimensions of the attractors in the second complex (6.0 for the L- and 7.6 for the H-chain) are somewhat larger than that of the first, probably reflecting the lower degree of order of the latter. In all cases, the satn. of the integral correlation coeff. with increasing no. of phase-space dimensions strongly indicates the existence of an attractor. The evidence of attractors in the **mol.** dynamics of proteins raises doubt about the value of trajectories calcd. by integration of equations of at. movement to the prediction of folding pathways since the stochastic element in the dynamics can eliminate leading equations in the set, thus influencing the folding pathway.

L43 ANSWER 2 OF 10 MEDLINE DUPLICATE 1
96234082 Document Number: 96234082. Novel unconventional **binding** site in the variable region of immunoglobulins. Rajagopalan K; Pavlinkova G; Levy S; Pokkuluri P R; Schiffer M; Haley B E; Kohler H. (Division of Medicinal Chemistry and Pharmaceutics, College of Pharmacy, Stanford University School of Medicine, CA 94305, USA.)PROCEEDINGS OF THE NATIONAL
ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Jun 11) 93 (12)
6019-24. Journal code: PV3. ISSN: 0027-8424. Pub. country: United States.
Language: English.
AB The variable immunoglobulin (Ig) domains contain hypervariable regions that are involved in the formation of the **antigen binding site**. Besides the canonical **antigen binding site**, so-called unconventional sites also reside in the variable region that **bind** bacterial and viral proteins. Docking to these unconventional sites does not typically interfere with antigen **binding**, which suggests that these sites may be a part of the biological functions of Igs. Herein, a novel unconventional **binding** site is described. The site is **detected** with 8-azidopurine nucleotide photoaffinity probes that label antibodies efficiently and under mild conditions. Tryptic peptides were isolated from
photolabeled monoclonal antibodies and aligned with the variable antibody domains of heavy and **light chains**. The structure of a variable Ig fragment was used to model the **binding** of the purine nucleotide to invariant residues in a hydrophobic pocket of the Ig **molecule** at a location distant from the **antigen binding site**. Monoclonal and polyclonal antibodies were biotinylated with the photoaffinity linker and used in fluorescence-activated cell sorter and ELISA analyses. The data support the utility of this site for tethering diagnostic and therapeutic agents to the variable Ig fragment region without impairing the structural and functional integrity of antibodies.

L43 ANSWER 3 OF 10 CAPLUS COPYRIGHT 1999 ACS
1997:104161 Document No. 126:156243 Apparent superoxide dismutase-like activity of immunoglobulin. Petyaev, I. M.; Hunt, J. V. (Division of Cellular Pathology, Department of Pathology, University of Cambridge, Cambridge, UK). Redox Rep., 2(6), 365-372 (English) 1996. CODEN: RDRPE4.
ISSN: 1351-0002. Publisher: Churchill Livingstone.
AB Using various superoxide generating systems and nitroblue tetrazolium or cytochrome c as superoxide **detector mols**. it is possible to assess the superoxide dismutase activity of proteins. Intact antibodies raised to different antigens, the Fab' fragment of anti-TNF [M632] and well-characterized recombinant Fv fragments of the murine antibody NQ11.7.22 appear to possess superoxide dismutase (SOD)-like activity. Kinetic characteristics of the SOD-like activity of

NQ11.7.22-Fv fragments suggest an enzymic property and these fragments behave in an analogous manner to human erythrocyte Cu-Zn SOD. Furthermore, the SOD-like activity of the NQ11.7.22-Fv fragment is affected by certain single-point mutations in the **amino acid** compn. and has a pH optimum of 6.2-6.6 which is unlike Cu-Zn SOD (pH 7.8-8.2). A change in tyrosine at the 32 position in the heavy chain and histidine at position 27 of the **light chain** of the NQ11.7.22-Fv fragment results in a profound redn. in SOD-like activity. Tyrosine at the 32 position in the heavy chain is known to play a significant role in antigen **binding** suggesting that the SOD-like activity occurs at the **antigen-binding site** itself. Single-point mutations at the periphery of the antigen combining site on the NQ11.7.22-Fv fragment had little or no effect on SOD-like activity. Further studies show that Ig (IgG1), a com. available murine monoclonal antibody, can also enhance the generation of hydrogen peroxide, the product of superoxide dismutation, when present in superoxide producing systems. The generation of hydrogen peroxide was increased by low pH (pH 6.25) with IgG1 but reduced with Cu-Zn SOD.

L43 ANSWER 4 OF 10 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1992-349209 [42] WPIDS
AB WO 9216624 A UPAB: 19931115

The following are claimed (A) monoclonal antibodies which recognise an epitope in the core region of the bacterial lipopolysaccharide (LPS) **mol.** and which are cross-protective against endotoxaemia caused by at least two different Gram -negative bacterial strains having different core structures, (B) hybridoma cell lines producing antibodies of type (A), (C) LPS- **binding** proteins having at least one **antigen-binding site** comprising at least one domain which comprises in sequence, the hypervariable hCDR1, hCDR2 and hCDR3 regions, where hCDR1 has the sequence Asp-Tyr-Tyr-Met-Thr, hCDR2 has the sequence Leu-Ile-Arg-Asn-W-Arg-Asn-Gly-Asp-Thr-Ala-Glu-Tyr-Ser-Ala-Ser-Val-X (W = Lys or Tyr, X = Lys or Arg) and hCDR3 has the sequence Gln-Gly-Arg-Gly-Tyr-Thr-Leu-Asp-Tyr, (D) DNA constructs coding for hCDR1, hCDR2 and hCDR3 in sequence, (E) DNA constructs encoding a heavy chain or fragment and comprising (a) a first part encoding a variable domain comprising alternate framework and hypervariable (hCDR1-hCDR2-hCDR3) regions, and (b) a second part encoding a heavy chain constant part or fragment, followed by a non-sense codon, (F) DNA constructs coding for the hypervariable regions lCDR1, lCDR2 and lCDR3 in sequence, where lCDR1 has the sequence Arg-Ala-Y-Z-Asn-Ile-Asn-Ile-Trp-Leu-Ser (Y = Ser or Arg, Z = Gln or Leu), lCDR2 has the sequence Lys-Ala-Ser-Asn-Leu-His-Thr and lCDR3 has the sequence Leu-Gln -Gly-Gln-Ser-Tyr-Pro-Arg-Thr (G) DNA constructs encoding a **light chain** or fragment and comprising (a) a first part encoding a variable domain comprising alternate framework and hypervariable (lCDR1-lCDR2-lCDR3) regions, and (b) a second part encoding a **light chain** constant part or fragment, followed by a non-sense codon.

USE - The antibodies and LPS-**binding** proteins are useful for prevention or treatment of Gram-negative endotoxaemia. The proteins are also useful for removing LPS from biological fluids by affinity chromatography. The labelled proteins are also useful for diagnostic purposes, e.g. for localising sites of infection or **detecting** bacterial contamination of water, foods etc. The DNA constructs may be used to produce humanised forms of the antibodies

Dwg.0/7

L43 ANSWER 5 OF 10 MEDLINE DUPLICATE 2
90062074 Document Number: 90062074. Protein L: an immunoglobulin **light chain-binding** bacterial protein.
Characterization of **binding** and physicochemical properties.
Akerstrom B; Bjorck L. (Department of Medical and Physiological Chemistry,
University of Lund, Sweden.)JOURNAL OF BIOLOGICAL CHEMISTRY, (1989 Nov 25) 264 (33) 19740-6. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Protein L, a cell wall **molecule** of the bacterial species Peptostreptococcus magnus with affinity for immunoglobulin (Ig) **light chains**, was isolated after solubilization of the bacterial cell walls with mutanolysin or from the culture medium by a single affinity chromatography step on human IgG-Sepharose. A major protein band with an apparent molecular weight of 95,000 was obtained from
both sources. The protein from the growth medium was size heterogeneous. From 1 ml of packed bacteria was prepared 0.92 mg of the mutanolysin-solubilized protein L (73% yield), whereas 4.1 mg of spontaneously released protein L (49% yield) was purified from the corresponding culture medium. The Mr of protein L was estimated to 76,000 by gel chromatography in 6 M guanidine HCl. Using this Mr value, the Stokes radius and the frictional ratio of protein L were determined to 4.74 nm and 1.70, respectively, suggesting an elongated fibrous **molecule**. No disulfide bond or subunit structure could be shown. The amino-terminal **amino acid** sequences of the whole protein and two internal non-IgG-**binding** tryptic fragments were determined and found to be unique. One of the tryptic fragments showed homology (40% identical residues) to a sequence within the cell wall-**binding** region of protein G, the Fc-**binding** protein of group C and G streptococci. The **binding** specificity of protein L was directed to the **light chains** of immunoglobulins; the affinity constant for polyacrylamide-coupled kappa-chains was 1.5 x 10(9) M-1 and for IgG, IgA, and IgM around 1 x 10(10) M-1. Maximal **binding** was achieved between pH 7 and 10. The **binding** to lambda-chains was too weak for determination of the affinity constant. 125I-Protein L was also shown to **bind** to mouse immunoglobulins. It could be used for **detection** of antigen-bound polyclonal and monoclonal antibodies in Western blots. This shows that the protein L/**light chain** reaction was not obstructed by occupation of the **antigen-binding site**. Finally, protein L and kappa-chains of human Ig formed precipitates upon double immunodiffusion analysis, an indication of at least two **binding** sites on protein L.

L43 ANSWER 6 OF 10 BIOSIS COPYRIGHT 1999 BIOSIS
1988:91311 Document No.: BA85:48083. CLINICAL UPDATE CROSS-REACTIVE IDIOTYPES AND THE GENETIC ORIGIN OF RHEUMATOID FACTORS. SILVERMAN G J; FONG S; CHEN P P; CARSON D A. DEP. BASIC CLINICAL RES., SCRIPPS CLIN. AND RES. FOUNDATION, LA JOLLA, CALIF.. J CLIN LAB ANAL, (1987) 1 (1), 129-135. CODEN: JCANEM. ISSN: 0887-8013. Language: English.

AB Rheumatoid factors (RFs) are present in a wide spectrum of diseases, but the diversity of these antibodies may vary greatly according to the underlying disease process. To analyze the diversity of these **molecules**, we have recently developed in the Scripps laboratory three cross-reactive idiotypic reagents against human monoclonal rheumatoid factors. These reagents **detect** unique markers associated with the kappa **light chain** variable region of the **antigen binding site** of these **molecules**. We have characterized the expression of the three cross-reactive idiotypes in serum RFs of patients with rheumatoid

arthritis. Sjogren's syndrome, and seropositive elderly. The autoantibodies from Sjogren's syndrome patients and from normal patients were restricted in heterogeneity and frequently displayed two or more of the cross-reactive idiotypes. In contrast, the RFs from rheumatoid arthritis patients expressed only one of the three cross-reactive idiotypic markers, suggesting greater structural diversity. The presence of shared idiotypes implies a common origin of the **light chains** from a single **light chain** gene or closely related genes. Cross-reactive idiotypes are useful diagnostic tools that distinguish the rheumatoid factors from different clinical processes and may provide insights concerning disease pathogenesis.

L43 ANSWER 7 OF 10 MEDLINE

85008196 Document Number: 85008196. Idiotypic analysis of anti-I-Ak monoclonal antibodies. I. Production and characterization of syngeneic anti-idiotypic mAb against an anti-I-Ak mAb. Phillips M L; Harris J F; Delovitch T L. JOURNAL OF IMMUNOLOGY, (1984 Nov) 133 (5) 2587-94. Journal code: IFB. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB To analyze the idiotype (Id) of anti-Ia antibodies elicited during alloimmune responses, we produced syngeneic mouse anti-Id monoclonal antibodies (mAb) reactive with the Id of the 11-5.2.1.9 (11-5) mouse anti-I-Ak (BALB/c anti-CKB) mAb. Two such anti-Id mAb, IA2 (IgG2a) and IIID1 (IgG1), **detect** structurally related idiotopes located within the **binding** site of 11-5 for I-Ak antigens. A third anti-Id mAb, VC6 (IgG1), **detects** an idiotope located either inside or outside of, but presumably proximal to, the 11-5 **antigen -binding site**, because its expression correlates with the antigenic specificity of 11-5. None of the idiotopes **detectable** by these three anti-Id mAb are accessible when the **binding** site of 11-5 is occupied by an I-Ak **molecule**. The association constants of these anti-Id mAb for their cognate Fab-linked Id range from 2 X 10(9) to 1 X 10(10) M-1. The three anti-Id-producing hybridomas were found with a frequency of 0.008% among growing hybrid colonies. Even though these anti-Id mAb **detect** public idiotopes (IdX) on 11-5, they do not **detect** the presence of such IdX markers in the sera of five syngeneic BALB/c mice hyperimmunized with C3H (I-Ak) spleen cells. This suggests that 11-5 represents a BALB/c idiotype infrequently expressed by serum immunoglobulins. The 11-5 idiotopes **detectable** by IA2, IIID1, and VC6 seem to be conformationally determined by the interaction of 11-5 H and L chains and are not confined to one or the other of these subunit polypeptides. Thus, the expression of the 11-5 Id may be regulated by both VH and VL genes.

L43 ANSWER 8 OF 10 MEDLINE DUPLICATE 3

84088933 Document Number: 84088933. Analysis of surface mu-chain expression in human lymphoblastoid cell lines that do not produce **light chains**. Hendershot L; Levitt D. JOURNAL OF IMMUNOLOGY, (1984 Jan) 132 (1) 502-9. Journal code: IFB. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB It has been suggested that **light chains** (LC) are necessary for the surface expression of mu heavy chains. Fluorescent antibody screening of 42 human lymphoblastoid cell lines transformed in our laboratory, however, disclosed four lines that expressed surface mu-chains without LC. The biosynthesis, glycosylation, and turnover of mu-chains in these cell lines was compared to mu-chain production in cell lines synthesizing both heavy chains and LC. LC production could not be **detected** in the mu +LC- cell lines by either surface or

biosynthetic labeling. The mu-chains expressed on the surface of the LC- cells appeared as disulfide-linked dimers and migrated slightly faster on SDS-polyacrylamide gels (70 Kd) than did mu-chains from IgM monomers (H2 L2) (78 Kd) after reduction. Biosynthetic labeling in the presence of tunicamycin demonstrated that the smaller size of free mu heavy chains was due to incomplete glycosylation of these **molecules** and not to **amino acid** deletions. The mu-chains produced by mu + LC- cells lines were degraded faster than mu-chains from LC+ cell lines, but their rate of transit to the cell surface was identical in both cell types. Thus, although LC are necessary for the formation of an intact **antigen-binding site**, they are not involved in the synthesis or expression of membrane mu-chains.

L43 ANSWER 9 OF 10 CAPLUS COPYRIGHT 1999 ACS
1979:418186 Document No. 91:18186 Immunoglobulin carbohydrate requirement for formation of an IgG-IgG complex. Hymes, A. Jeanne; Mullinax, Grace Lane; Mullinax, Franklin (Med. Coll. Virginia, Virginia Commonwealth Univ., Richmond, VA, 23298, USA). J. Biol. Chem., 254(9), 3148-51 (English) 1979. CODEN: JBCHA3. ISSN: 0021-9258.

AB In addn. to their fragment Fc oligosaccharides, some Ig **mols.** have oligosaccharides linked to variable segments of heavy or light (H or L) chains. These fragment Fab oligosaccharides are potential determinants of antibody specificity. This possibility was considered in a study of the IgG antiglobulin from a patient with IgG-IgG complexes. F(ab')2 fragments of the antiglobulin retained the ability to form complexes with normal IgG as **detected** by anal. ultracentrifugation. Removal of F(ab')2 sialic acids by neuraminidase abolished complex formation. Recombination expts. further localized antiglobulin activity to the L chains. Antiglobulin activity of the recombinant **mols.** was shown by anal. ultracentrifugation and by column chromatog. with **mols.** contg. 125I-labeled L chains. L chains from the subject's IgG were enriched in sialic acids. Thus, a sialic acid-contg. oligosaccharide on the L chain of this antiglobulin is required for its **binding** action.

L43 ANSWER 10 OF 10 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 4
1974:94077 Document No. 80:94077 Affinity labeling of a distinctive lysyl residue within the second hypervariable region of .gamma.2 chain of guinea pig anti-p-azobenzenearsonate antibody. Koo, Peter H.; Cebra, John J. (Dep. Biol., Johns Hopkins Univ., Baltimore, Md., USA). Biochemistry, 13(1), 184-95 (English) 1974. CODEN: BICHAW.

AB Anti-p-azobenzenearsonate (anti-ARS) antibodies, purified from serums of inbred strain 13 guinea pigs, were affinity labeled with N-[1-14C]bromoacetylmono(p-azobenzenearsonic acid)-L-tyrosine (BAAT). Each mole of anti-ARS antibodies bound 1 mole of BAAT covalently, while only 0.08 mole of BAAT was bound per mole of nonspecific IgG2. The affinity labeling reaction could be inhibited by haptens such as p-nitrobenzenearsonate and the N.alpha.-acetyl analog of BAAT, preincubated with the antibodies. The proportion of label incorporated into heavy and **light chains** was estd. by Na dodecyl sulfate polyacrylamide gel electrophoresis to be 2.3:1. Lysine and tyrosine were the only major residues labeled in the whole antibody **mol.** and their labeled derivs. were recovered in a ratio of 1.6:1, resp. CNBr fragments C-1-n, C-1-a1, and C-1-a2 account for residues from N-1 to N-140 of .gamma.2 chain and include those residue positions which have different **amino acids** in antibodies of different antigen-**binding** specificities. Most of these variable residue positions occur in 3 short segments called hypervariable regions. The

fragments C-1-n, C-1-a1, and C-1-a2 were sepd. from a CNBr digest of affinity-labeled anti-ARS antibodies. About 94% of their total label was estd. to be localized in C-1-a1 (N-35 to N-83). The peptides from enzymic digests of C-1-a1, including a radio-labeled tripeptide, were isolated, partially sequenced, and aligned. The anti-ARS antibody, like anti-dinitrophenyl antibody, had a C-1-a1 with a distinctive primary structure of restricted heterogeneity, even within the 2nd hypervariable region. The only conspicuously labeled residue in C-1-a1 occurred at position N-59 and it could be released by automatic sequential degrdn. Although antibodies of different antigen-**binding** specificities have different **amino acids** at N-59, only a lysyl residue was **detected** there in anti-ARS antibodies, and radiolabeled carboxymethyl-lysine was identified at N-59 in affinity-labeled **mols**. The highly specific labeling of anti-ARS antibody in a normally variable position within a hypervariable region suggests that lysine N-59 may be a contact residue in the **antigen -binding site**. Thus, this very lysyl residue, distinctive for anti-ARS antibody, may both contribute to **binding** specificity for ligands such as BAAT and also be chem. modified by them.

=> s stevens f?/au,in;s schiffer m?/au,in;s wilkins stevens p?/au,in or stevens p?/au,in

'IN' IS NOT A VALID FIELD CODE
L44 214 FILE MEDLINE
L45 176 FILE CAPLUS
L46 235 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L47 154 FILE EMBASE
L48 27 FILE WPIDS

TOTAL FOR ALL FILES
L49 806 STEVENS F?/AU,IN

'IN' IS NOT A VALID FIELD CODE
L50 128 FILE MEDLINE
L51 142 FILE CAPLUS
L52 154 FILE BIOSIS
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L53 68 FILE EMBASE
L54 9 FILE WPIDS

TOTAL FOR ALL FILES
L55 501 SCHIFFER M?/AU,IN

'IN' IS NOT A VALID FIELD CODE
L56 412 FILE MEDLINE
L57 359 FILE CAPLUS
L58 592 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L59 325 FILE EMBASE
L60 36 FILE WPIDS

TOTAL FOR ALL FILES
L61 1724 WILKINS STEVENS P?/AU,IN OR STEVENS P?/AU,IN

=> s l49 and l55 and l61

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L62           3 FILE MEDLINE
L63           3 FILE CAPLUS
L64           5 FILE BIOSIS
L65           3 FILE EMBASE
L66           0 FILE WPIDS

TOTAL FOR ALL FILES
L67          14 L49 AND L55 AND L61

=> dup rem l67

PROCESSING COMPLETED FOR L67
L68           5 DUP REM L67 (9 DUPLICATES REMOVED)

=> d cbib abs 1-5
```

L68 ANSWER 1 OF 5 MEDLINE DUPLICATE 1

1999190074 Document Number: 99190074. Physicochemical consequences of amino acid variations that contribute to fibril formation by immunoglobulin light chains. Raffen R; Dieckman L J; Szpunar M; Wunschl C; Pokkuluri P R;
Dave P; **Wilkins Stevens P**; Cai X; **Schiffer M**;
Stevens F J. (Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory, Illinois 60439, USA.)PROTEIN SCIENCE, (1999 Mar) 8 (3) 509-17. Journal code: BNW. ISSN: 0961-8368. Pub. country: United States. Language: English.

AB The most common form of systemic amyloidosis originates from antibody light chains. The large number of amino acid variations that distinguish amyloidogenic from nonamyloidogenic light chain proteins has impeded our understanding of the structural basis of light-chain fibril formation. Moreover, even among the subset of human light chains that are amyloidogenic, many primary structure differences are found. We compared the thermodynamic stabilities of two recombinant kappa4 light-chain variable domains (V(L)s) derived from amyloidogenic light chains with a V(L) from a benign light chain. The amyloidogenic V(L)s were significantly less stable than the benign V(L). Furthermore, only the amyloidogenic V(L)s formed fibrils under native conditions in an in vitro fibril formation assay. We used site-directed mutagenesis to examine the consequences of individual amino acid substitutions found in the amyloidogenic V(L)s on stability and fibril formation capability. Both stabilizing and destabilizing mutations were found; however, only destabilizing mutations induced fibril formation in vitro. We found that fibril formation by the benign V(L) could be induced by low concentrations of a denaturant. This indicates that there are no structural or sequence-specific features of the benign V(L) that are incompatible with fibril formation, other than its greater stability. These studies demonstrate that the V(L) beta-domain structure is vulnerable to destabilizing mutations at a number of sites, including complementarity determining regions (CDRs), and that loss of variable domain stability is a major driving force in fibril formation.

L68 ANSWER 2 OF 5 MEDLINE DUPLICATE 2

1998416701 Document Number: 98416701. A domain flip as a result of a single amino-acid substitution. Pokkuluri P R; Huang D B; Raffen R; Cai X; Johnson G; **Stevens P W; Stevens F J; Schiffer M.** (Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory, IL 60439, USA.)STRUCTURE, (1998 Aug 15) 6 (8)

1067-73. Journal code: B31. ISSN: 0969-2126. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: The self-assembly properties of beta domains are important features of diverse classes of proteins that include cell-adhesion molecules, surface receptors and the immunoglobulin superfamily. Immunoglobulin light-chain variable domains are well suited to the study of structural factors that determine dimerization, including how residues at the interface influence the preferred dimer arrangement. RESULTS: Single-site mutants of light-chain variable domain Len, designated LenQ38E and LenK30T, formed 'flipped' dimers in which one domain was rotated by about 180 degrees compared with the native protein. The dimer in the native protein is similar to that found between variable domains in Fab immunoglobulin fragments. When compared to the native dimer, more surface area is buried, and more hydrogen bonds and salt bridges are formed between the monomers in the flipped conformation. CONCLUSIONS: Immunoglobulin light-chain variable domains can form a minimum of two distinct quaternary structures. Single-site mutations resulting from changes of one base, such as the exchange of Gln38 to Glu or Lys30 to Thr, change the 'conventional' dimer of protein Len to a flipped arrangement. Native Len is not found in the flipped-domain dimer conformation because it would have excess positive electrostatic potential at the dimer interface that is not compensated by other forces. Excess negative or positive electrostatic potential at the dimer interface can have a determining effect on the mode of dimerization.

L68 ANSWER 3 OF 5 MEDLINE DUPLICATE 3

1998343786 Document Number: 98343786. Reengineering immunoglobulin domain interactions by introduction of charged residues. Raffen R; **Stevens P W**; Boogaard C; **Schiffer M**; **Stevens F J.** (Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory, IL 60439, USA.)PROTEIN-ENGINEERING, (1998 Apr) 11 (4) 303-9. Journal code: PR1. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The formation of the antibody variable domain binding unit (Fv) is the net result of three competing assembly reactions. The affinities of concurrent homologous interactions of heavy and light chain variable domains limits the heterologous interaction leading to productive formation of the Fv. To address the possible role of light chain dimerization in this phenomenon, the Gln38 residue at the dimer interface of an immunoglobulin light chain variable domain (VL) was replaced by charged amino acids. The effects of these mutations on VL homodimer formation were monitored by small-zone size exclusion HPLC and the affinities of interaction were determined by computer simulation. Reduced VL homodimerization was observed in three of the four mutants, Q38R, Q38D and Q38K. The association constants for the Q38R and Q38D homodimers were 1.2 x 10(4) and 3.2 x 10(3) M(-1), respectively. This corresponded to a 20-75-fold reduction in the homodimer association constant relative to the wild-type VL, which had an association constant of 2.4 x 10(5) M(-1). Surprisingly, the fourth charge mutant, Q38E, had a higher association constant than the wild-type VL. The potential for charged residues to facilitate heterodimeric assembly of immunoglobulin domains was also tested. Heterodimerization was observed between the Q38D and Q38R V(L)s, but with an association constant of 4.7 x

10(4) M(-1), approximately fivefold lower than that obtained for homodimerization of the native V(L). In addition, replacement of the neutral, solvent-accessible Gln38 residue with either Asp or Arg was found to be significantly destabilizing. These results suggest that charged residues could be introduced at immunoglobulin domain interfaces to guide heterodimer formation and to minimize unfavorable competing homologous associations. Nonetheless, these apparently simple modifications may also result in unintended consequences that are likely to depend upon structural features of particular variable domains.

L68 ANSWER 4 OF 5 BIOSIS COPYRIGHT 1999 BIOSIS
1996:140214 Document No.: PREV199698712349. In vitro characterization of light chain amyloidosis using recombinant light chain variable domains. Raffen, R.; **Stevens, P. Wilkins**; Hanson, D. K.; Deng, Y.; Berrios-Hammond, M.; Westholm, F. A.; **Schiffer, M.**; **Stevens, F. J.**. Argonne National Lab., Argonne, IL 60439 USA. Biophysical Journal, (1996) Vol. 70, No. 2 PART 2, pp. A65. Meeting Info.: 40th Annual Meeting of the Biophysical Society Baltimore, Maryland, USA February 17-21, 1996 ISSN: 0006-3495. Language: English.

L68 ANSWER 5 OF 5 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 4
1995:220686 Document No.: PREV199598234986. Recombinant immunoglobulin variable domains generated from synthetic genes provide a system for in vitro characterization of light-chain amyloid proteins. **Stevens, Priscilla Wilkins**; Raffen, Rosemarie; Hanson, Deborah K.; Deng, Ya-Li; Berrios-Hammond, Maria; Westholm, Florence A.; Murphy, Charles; Eulitz, Manfred; Wetzel, Ronald; Solomon, Alan; **Schiffer, Marianne**; **Stevens, Fred J.** **(1)**. (1) Cent. Mechanistic Biol. Biotechnol., Argonne Natl. Lab., Argonne, IL 60439 USA. Protein Science, (1995) Vol. 4, No. 3, pp. 421-432. ISSN: 0961-8368. Language: English.

AB The primary structural features that render human monoclonal light chains amyloidogenic are presently unknown. To gain further insight into the physical and biochemical factors that result in the pathologic deposition of these proteins as amyloid fibrils, we have selected for detailed study three closely homologous protein products of the light-chain variable-region single-gene family V-kappa-IV. Two of these proteins, REC and SMA, formed amyloid fibrils in vivo. The third protein, LEN, was excreted by the patient at levels of 50 g/day with no indication of amyloid deposits. Sequences of amyloidogenic proteins REC and SMA differed from the sequence of the nonpathogenic protein LEN at 14 and 8 amino acid positions, respectively, and these amino acid differences have been analyzed in terms of the three-dimensional structure of the LEN dimer. To provide a replenishable source of these human proteins, we constructed synthetic genes coding for the REC, SMA, and LEN variable domains and expressed these genes in Escherichia coli. Immunochemical and biophysical comparisons demonstrated that the recombinant V-kappa-IV products have tertiary structural features comparable to those of the patient-derived proteins. This welldefined set of three clinically characterized human kappa-IV light chains, together with the capability to produce these kappa-IV proteins recombinantly, provide a system for biophysical and structural comparisons of two different amyloidogenic light-chain proteins and a nonamyloidogenic protein of the same subgroup. This work lays the foundation for future investigations of the structural basis of light-chain amyloidogenicity.

7 ANSWER 8 OF 9 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
AN 95102716 EMBASE
DN 1995102716
TI **Recombinant** immunoglobulin variable domains generated from **synthetic genes** provide a system for in vitro characterization of **light-chain** amyloid **proteins**.
AU Stevens P.W.; Raffen R.; Hanson D.K.; Deng Y.-L.; Berrios-Hammond M.; Westholm F.A.; Murphy C.; Eulitz M.; Wetzel R.; Solomon A.; Schiffer M.; Stevens F.J.
CS Argonne National Laboratory, Mechanistic Biology/Biotechnol. Ctr.,Argonne, IL 60439, United States
SO Protein Science, (1995) 4/3 (421-432).
ISSN: 0961-8368 CODEN: PRCIEI
CY United States
DT Journal; Article
FS 005 General Pathology and Pathological Anatomy
029 Clinical Biochemistry
LA English
SL English
AB The primary structural features that render human monoclonal **light chains** amyloidogenic are presently unknown. To gain further insight into the physical and biochemical factors that result in the pathologic deposition of these **proteins** as amyloid fibrils, we have selected for detailed study three closely homologous **protein** products of the **light-chain** variable-region single-**gene** family V.kappa.IV. Two of these **proteins**, REC and SMA, formed amyloid fibrils in vivo. The third **protein**, LEN, was excreted by the patient at levels of 50 g/day with no indication of amyloid deposits. Sequences of amyloidogenic **proteins** REC and SMA differed from the sequence of the nonpathogenic **protein** LEN at 14 and 8 amino acid positions, respectively, and these amino acid differences have been analyzed in terms of the three- dimensional structure of the LEN **dimer**. To provide a replenishable source of these human **proteins**, we constructed **synthetic genes** coding for the REC, SMA, and LEN variable domains and expressed these **genes** in Escherichia coli. Immunochemical and biophysical comparisons demonstrated that the **recombinant** V.kappa.IV products have tertiary structural features comparable to those of the patient-derived **proteins**. This well-defined set of three clinically characterized human .kappa.IV **light chains**, together with the capability to produce these .kappa.IV **proteins** recombinantly, provide a system for biophysical and structural comparisons of two different amyloidogenic **light-chain proteins** and a nonamyloidogenic **protein** of the same subgroup. This work lays the foundation for future investigations of the structural basis of **light- chain** amyloidogenicity.
CT Medical Descriptors:
*amyloidosis
amino acid sequence
article
human
priority journal
protein determination
sequence analysis
structure analysis
Drug Descriptors:
*amyloid
***immunoglobulin light chain**
RN (amyl

L8 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2000 ACS
AN 1997:215733 CAPLUS
DN 126:196107
TI Manufacture of multimeric proteins, especially insulin, with recombinant organisms
IN Hadfield, Christopher; Meacock, Peter Anthony; Krishnaswamy, Patnam Rajagopala; Shashi, Kaithamana; Raina, Krishna Kumar; Ramadoss, Candadai Seshadri
PA University of Leicester, UK; Vittal Mallya Scientific Research Foundation; Hadfield, Christopher; Meacock, Peter Anthony; Krishnaswamy, Patnam Rajagopalalyengar; Shashi, Kaithamana; Raina, Krishna Kumar; Ramadoss, Candadai Seshadri
SO PCT Int. Appl., 96 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C07K
ICS A61K
CC 3-2 (Biochemical Genetics)
Section cross-reference(s): 16
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9703089	A2	19970130	WO 1996-GB1620	19960708
WO 9703089	A3	19970313		
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA				
AU 9663144	A1	19970210	AU 1996-63144	19960708
PRAI GB 1995-13967		19950708		
WO 1996-GB1620		19960708		

AB The present invention concerns a protein precursor for at least two polypeptide chains having the general formula B-Z-A wherein B and A are the two polypeptide chains of a **double-chain mol.**, the two chains being linked by at least one disulfide bond, and Z is a polypeptide comprising at least one proteolytic cleavage site. Also provided are DNA sequences encoding same, organisms transformed and transfected with same, and methods for the prodn. of the **double-chain mol.** Numerous insulin precursor genes were prepd. which encoded the B and A chains of human, porcine, or bovine insulin connected by linkers providing protease or CNBr cleavage sites as well as peptides useful for purifn., e.g. a myc epitope. The codons were optimized for expression in yeast. Yields of up to 2.0 mg/L were obtained.
ST multimeric protein precursor recombinant; insulin precursor recombinant Saccharomyces fermn
IT DNA sequences
(for mammalian insulin precursors)
IT Eukaryote (Eukaryotae)
Fungi
Saccharomyces cerevisiae
Yeast
(manuf. of multimeric proteins, esp. insulin, with recombinant organisms)
IT Proteins (specific proteins and subclasses)
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(multimeric; manuf. of multimeric proteins, esp. insulin, with recombinant organisms)
IT Protein sequences
(of mammalian insulin precursors)
IT 187855-05-6P 187855-06-7P 187855-07-8P 187855-08-9P 187887-86-1P 187887-87-2P 187887-88-3P 187887-89-4P 187887-90-7P
RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(amino acid sequence; manuf. of multimeric proteins, esp. insulin, with recombinant organisms)
IT 9004-10-8P, Insulin, preparation 11061-68-0P, Insulin (human)
11070-73-8P, Insulin (bovine) 12584-58-6P, Insulin (swine)

L7 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2000 ACS
AN 1993:493518 CAPLUS
DN 119:93518
TI Production of chimeric antibodies - a combinatorial approach
IN Hoogenboom, Hendricus Renerus Jacobus Matteus; Baier, Michael; Jespers, Laurent Stephane Anne Therese; Winter, Gregory Paul
PA Medical Research Council, UK; Cambridge Antibody Technology Ltd.
SO PCT Int. Appl., 107 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C12N015-00
ICS C07K013-00; C12N015-13; C12N015-62
CC 15-3 (Immunochemistry)
Section cross-reference(s): 3
FAN.CNT 6

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9306213	A1	19930401	WO 1992-GB1755	19920923
	W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG				
	WO 9220791	A1	19921126	WO 1992-GB883	19920515
	W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, US				
	RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GN, GR, IT, LU, MC, ML, MR, NL, SE, SN, TD, TG				
	AU 9225933	A1	19930427	AU 1992-25933	19920923
	AU 665025	B2	19951214		
	EP 605522	A1	19940713	EP 1992-919846	19920923
	EP 605522	B1	19990623		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, SE				
	AT 181571	E	19990715	AT 1992-919846	19920923
	ES 2136092	T3	19991116	ES 1992-919846	19920923
	WO 9311236	A1	19930610	WO 1992-GB2240	19921202
	W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG				
	AU 9230890	A1	19930628	AU 1992-30890	19921202
	AU 665221	B2	19951221		
	EP 616640	A1	19940928	EP 1992-924775	19921202
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	EP 1024191	A2	20000802	EP 2000-107845	19921202
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE				
	WO 9319172	A1	19930930	WO 1993-GB605	19930324
	W: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML				
	AU 9337638	A1	19931021	AU 1993-37638	19930324
	AU 673515	B2	19961114		
	CA 2131151	AA	19940930	CA 1993-2131151	19930324
	JP 07505055	T2	19950608	JP 1993-516400	19930324
	EP 656941	A1	19950614	EP 1993-906742	19930324
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	US 5565332	A	19961015	US 1994-211202	19940624
	US 5858657	A	19990112	US 1995-480006	19950607

PRAI	GB 1991-20252	19910923
	GB 1991-20377	19910925
	GB 1992-6318	19920324
	GB 1992-6372	19920324
	WO 1992-GB883	19920515
	GB 1991-10549	19910515
	WO 1991-GB1134	19910710
	GB 1991-25579	19911202
	GB 1991-25582	19911202
	GB 1992-883	19920515
	WO 1992-GB1755	19920923
	EP 1992-924775	19921202

from this library may be used in a 2nd humanizing shuffling step or modified to increase hum[redacted] character still further. Thus[redacted] a mouse antibody directed agains[redacted]n epitope of human tumor necro[redacted] factor .alpha. (TNF-.alpha.) was cloned as an Fab fragment for display on phage, and by combining the heavy chain with repertoires of human **light chains** (or by combining the **light chain** with repertoires of human heavy chains), it was possible to select phage bearing Fab fragments with 1 mouse and 1 human chain. These antibody fragments bound to the same epitope of TNF-.alpha. as the original mouse antibody. The new human chain (heavy or light) was then combined with a repertoire of human partner chains to create an entirely human antibody Fab fragment which bound to the same epitope of TNF-.alpha..

ST chimeric **recombinant** antibody chain shuffling; humanized antibody prodn chain shuffling

IT Genetic vectors
(chimeric **recombinant** antibody **genes** on, antibody humanization by chain shuffling and complementarity-detg. region imprinting in relation to)

IT Antibodies
RL: BIOL (Biological study)
(chimeric **recombinant**, humanization of, chain shuffling and complementarity-detg. region imprinting in)

IT **Gene**, animal
RL: BIOL (Biological study)
(for chimeric **recombinant** antibody, antibody humanization by chain shuffling and complementarity-detg. region imprinting in relation to)

IT **Protein** sequences
(of chimeric **recombinant** antibodies to human immunodeficiency virus glycoprotein gp120 and tumor necrosis factor .alpha.)

IT Deoxyribonucleic acid sequences
(complementary, for chimeric **recombinant** antibodies to human immunodeficiency virus glycoprotein gp120 and tumor necrosis factor .alpha.)

IT Virus, bacterial
(fd, chimeric **light chain** of **recombinant** monoclonal antibody to tumor necrosis factor .alpha. expression on, humanization in relation to)

IT Sialoglycoproteins
RL: BIOL (Biological study)
(gp120env, of human immunodeficiency virus, chimeric **recombinant** antibody to, humanization of, by chain shuffling and complementarity-detg. region imprinting)

IT Virus, animal
(human immunodeficiency, glycoprotein gp120 of, chimeric **recombinant** antibody to, humanization of, by chain shuffling and complementarity-detg. region imprinting)

IT Antibodies
RL: BIOL (Biological study)
(monoclonal, to tumor necrosis factor .alpha., humanization of, by chain shuffling and complementarity-detg. region imprinting)

IT Genetic vectors
(phagemid, pHEN1, **gene** for monoclonal antibody to tumor necrosis factor .alpha. cloning in, for humanization by chain shuffling and complementarity-detg. region imprinting)

IT Lymphokines and Cytokines
RL: BIOL (Biological study)
(tumor necrosis factor-.alpha., monoclonal antibody to, humanization of, by chain shuffling and complementarity-detg. region imprinting)

IT 148325-38-6 148325-40-0
RL: PRP (Properties)
(amino acid sequence of and cloning of **gene** for, for humanization by chain shuffling)

IT 148325-36-4 148325-41-1 148325-42-2 148325-43-3 148325-44-4
148325-45-5 148325-46-6 148325-47-7 148325-48-8 148325-49-9
148325-50-2 148325-51-3 148325-52-4 148325-53-5 148325-54-6
148325-55-7 148325-56-8 148325-57-9 148325-58-0
RL: PRP (Properties)
(amino acid sequence of and cloning of **gene** for, humanization by chain shuffling in relation to)

IT 1199-01-5, 2-Phenyloxazol-5-one
RL: PRP (Properties)
(antibody to, prepn. of **synthetic**, by genetic engineering,

7 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2000 ACS
AN 1992:126478 CAPLUS
DN 116:126478
TI Characterization of a **recombinant** single-chain molecule comprising the variable domains of a monoclonal antibody specific for human fibrin fragment D-**dimer**
AU Laroche, Yves; Demeyer, Marc; Stassen, Jean Marie; Gansemans, Yannick; Demarsin, Eddy; Matthyssens, Gaston; Collen, Desire; Holvoet, Paul
CS Corvas Int. NV, Ghent, Belg.
SO J. Biol. Chem. (1991), 266(25), 16343-9
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
CC 15-3 (Immunochemistry)
Section cross-reference(s): 8
AB A **recombinant** single-chain mol., scFv-K12G0, contg. the variable domains of the monoclonal antibody MA-15C5, specific for fragment D-**dimer** of human cross-linked fibrin, was constructed and expressed in Spodoptera frugiperda, Sf9, insect cells. The Arg108 C-terminal amino acid of the variable domain of the **light-chain** of the antibody was connected through a **synthetic** Ala-Gly-Gln-GLy-Ser-Ser-Val peptide linker with the Gln1 N-terminal amino acid of the variable domain of its heavy chain. ScFv-K12G0 was secreted by the infected Sf9 cells at a rate of 10 .mu.g/106 cells within 48 h, resulting in conditioned medium with a max. concn. of 15 mg of scFv-K12G0/L. The mol., purified to homogeneity by ion exchange chromatog. and gel filtration, migrated as a single Mr band on reduced SDS-gel electrophoresis. It bound to immobilized fragment D-**dimer** with an affinity const. of 4.0 .times. 109 M-1 (2.0 .times. 1010 M-1 for intact MA-15C5). Clearing of scFv-K12G0 from the circulation in rabbits occurred with an initial half-life (t1/2.alpha.) of 10 min and a clearance of 5.1 mL min-1, as compared to 90 min and 210 mL min-1 for intact MA-15C5. Nephrectomy resulted in a prolongation of t1/2.alpha. to 110 min, suggesting that the rapid clearance of scFv-K12G0 occurs primarily via the kidney, presumably by glomerular filtration. Thus, the single-chain **recombinant** mol. scFv-K12G0 is secreted in functionally intact form and it may be useful for targeting of radioisotopes or plasminogen activators to blood clots in vivo.
ST single chain antibody fibrin fragment
IT **Gene**, animal
RL: BIOL (Biological study)
(for **recombinant** single-chain mol. comprising V domains of monoclonal antibody to human fibrin fragment D)
IT Deoxyribonucleic acid sequences
Protein sequences
(of **recombinant** single-chain mol. comprising V domains of monoclonal antibody to human fibrin fragment D)
IT Fibrinogen degradation products
RL: BIOL (Biological study)
(D, monoclonal antibody to human, **recombinant** single-chain mol. comprising variable domains of, characterization of)
IT Scintigraphy
(immuno-, of fibrin clots, single chain monoclonal antibody V domains construct to human fibrin D fragment in relation to)
IT Antibodies
RL: BIOL (Biological study)
(monoclonal, to fibrin fragment D, of humans, **recombinant** single-chain mol. comprising variable domains of, characterization of)
IT 139381-32-1, Immunoglobulin G (mouse clone pVLK12G0 scFv-K12G0 anti-human fibrinopeptide DD reduced) 139381-33-2, Immunoglobulin G (mouse clone pVLK12G0 scFv-K12G0 precursor anti-human fibrinopeptide DD reduced)
RL: PRP (Properties)
(amino acid sequence of)
IT 139382-55-1, Deoxyribonucleic acid (mouse clone pVLK12G0 scFv-K12G0 immunoglobulin G precursor-specifying) 139382-56-2
RL: PRP (Properties)
(nuc